Chemical group identification
Supplementary data

Alkaloid identification

The presence of alkaloid was determined using Dragendorff, Mayer and Wagner reagents. The Dragendorff solution (a) was prepared with 4.25 mg of bismuth nitrate (III) and 50 g of tartaric acid dissolved into 200 mL of water. The potassium iodide solution (b) was prepared using 80 g of iodine dissolved into 200 mL of water. Reagents were kept at 4 ºC until use. Mayer´s reagent (a) was prepared using 2.72 g of mercury chloride dissolved into 120 mL of water and (b): 10 g of potassium iodide dissolved into 20 mL of water. Both solutions were mixed and diluted with water until complete 200 mL. The Wagner reagent was prepared using 2.54 g of iodine and 4 g of potassium iodide dissolved into 10 ml of water. The solution was added with water until complete 200 mL. The technique to identify the presence of alkaloids was as follows: 50 mg of hydroalcoholic extract were put into a test tube and 6 mL of HCl (10%) were added. The mixture was heated for 10 min at 100 ºC. After that the tube was cooled and filtered. The solution was divided in three test tubes and 10 drops of the reagents (Dragendorff, Mayer, and Wagner reagents) were added. Precipitate and a light turbidity, as well as change in colour to red, orange, white or brown precipitate is considered as an evidence of the presence of alkaloids.

Volatile coumarins identification

A volume of three millilitres of the hydroalcoholic extract was placed into a test tube to assay for volatile coumarins. The test tube aperture was coated with a filter paper impregnated with 1N NaOH solution. The test tube was put on a water bath at 100 ºC for 10 min. The filter paper was observed

under ultraviolet light to visualise the presence of yellow-coloured fluorescence indicative of coumarins.

Flavonoid identification

Two mL of the hydroalcoholic extract were placed into a test tube and a small piece of magnesium band (5 x 5 mm) and 20 mL of hydrochloric acid (36%) were added and incubated at room temperature (25-28 ºC). After 24 h a colour change (red) indicated flavonoids.

Tannins identification

In order to identify the presence of tannins, the following solutions were prepared: (a) 20% ferric chloride solution; (b) 1% gelatine solution; (c) gelatine and salt solution (500 mg of gelatine and 5 g of sodium chloride in 50 mL of distilled water); (d) 10% saline solution. The presence of tannins in P. laevigata was determined by dissolving 100 mg of hydroalcoholic extract in 10 mL of distilled water, and 3 mL of the resulting extract was placed into a test tube and 1 to 3 drops of 20% ferric chloride solution were added. In order to confirm the presence of tannins the remained extract was divided in three equal parts of 10 mL. Into each test tube 1 mL of each gelatine solution, gelatine solution + salt, and saline solution was added. The presence of a precipitate into the tubes with gelatine solution and gelatine + salt indicates the presence of tannins. A negative result is obtained when a precipitate formation is observed into the test tube with saline solution.

Triterpene and steroid identification

In order to identity the presence of triterpenes and steroids in a 30/100 mm glass test tube 50 mg of the hydroalcoholic extract were solubilized into the test tube using 10 mL of chloroform, and then filtered using Whatman paper (No. 4). The solution was divided into two test tubes to react with the Liebermann-Burchard and the Salkowski reagents. The first was prepared using 1 mL of acetic anhydride to which 0.5 mL of sulphuric acid was added, handling the solution in an ice bath; and the Salkowski reagent consisted of concentrated sulphuric acid (0.5 mL). Both solutions were added individually to each tube containing the chloroformic phase. The change of colour to yellow/red was considered as positive to the presence of triterpenes or steroids.

Saponins identification

The presence of saponins was determined as follows: 5 mL of the hydroalcoholic extract were deposited into a test tube, and then placed in a water bath to boil for 2 minutes. After cool, the tube was vigorously shaken to form persistent foam that was positive for saponins.